Poster Abstracts

On-site poster session 1

9th May 3 - 4 pm

Biofilm ecology and ecotoxicology

Biofilm application

On-site

Poster session: Biofilm ecology and ecotoxicology

Permissive aggregative group formation favors coexistence in yeast

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n Saccharomyces cerevisiae, the FLO1 gene encodes flocculins that lead to formation of biofilm-like, multicellular flocs, that offer protection to the constituent cells. Flo1p was found to preferentially bind to fellow cooperators compared to defectors lacking FLO1 expression, resulting in enrichment of cooperators within the flocs. Given this dual function in cooperation and kin recognition, FLO1 has been termed a 'green beard gene'. Because of the heterophilic nature of Flo1p binding however, we hypothesize that kin recognition is permissive and depends on the relative stability of FLO1+/flo1− versus FLO1+/FLO1+ bonds, which itself can be dependent on environmental conditions and intrinsic cell properties. We combine single cell measurements of adhesion strengths, individual cell-based simulations of cluster formation and evolution, and in vitro flocculation experiments to study the impact of relative bond stability on defector exclusion as well as benefit and stability of cooperation. We hereto vary the relative bond stability by changing the shear flow rate and the inherent bond strength. We identify a marked trade-off between both aspects of the green beard mechanism, with reduced relative bond stability leading to increased kin recognition, but at the expense of decreased cluster sizes and benefit of cooperation. Most notably, we show that the selection of FLO1 cooperators is negative-frequency dependent, which we directly attribute to the permissive character of the Flo1p bond. Taking into account the costs associated to FLO1 expression, this asymmetric selection results in a broad range of ecological conditions where coexistence between cooperators and defectors is stable. Although the kin recognition aspect of the FLO1 'green beard gene' is thus limited and condition dependent, the negative-frequency dependency of selection can conserve the diversity of flocculent and non-flocculent phenotypes ensuring flexibility towards variable selective pressures.

On-site

Poster session: Biofilm ecology and ecotoxicology

Grazer control of autotrophic stream biofilms – food quality matters

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Autotrophic biofilms in streams are massively stimulated by enhanced nutrient loading ("bottom-up control") resulting in eutrophication. However, even under high nutrient loading, autotrophic biofilms can be "top-down" controlled by grazers. While grazing is potentially an alternative control mechanisms of eutrophication, the regulation of grazing itself (i.e., why grazers have a strong effect in some ecosystems, but weak effects in others) is yet poorly understood. Here we tested the hypothesis that the strength of top-down control of algal biofilms is determined by the food quality of the algae, which is in turn regulated by allocation of essential resources. We tested this hypothesis on different scales and complexity levels in both highly controlled laboratory experiments and field-related mesocosm experiments. This includes the homogenous local patch size, the multi-patch level with spatial heterogeneity and the choice for gazers as well as high-complexity level under consideration of growth and migration behaviour of grazers in mesocosm experiments. Our data show that anthropogenic eutrophication leads to a reduction of top-down control exerted by herbivores via both alterations in grazing activity and spatial structuring of the biofilms. This demonstrates how the strength of top-down pressure on biofilms is regulated and contributes to our understanding of eutrophication control in natural surface waters.

On-site

Poster session: Biofilm ecology and ecotoxicology

Biofilm formation diversity of Brochothrix thermosphacta a major food spoilage

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Introduction: Biofilms play an important role in residence and persistence of microorganisms in food industry. Brochothrix thermosphacta is considered as a major food spoilage bacteria (Illikoud et al., 2019). This bacteria has been identified in biofilms on multiple surfaces of the food processing environment (Wagner et al., 2021, 2020).

Purpose: The study of B.thermosphacta ability to form biofilm is necessary to better understand the residence and persistence of this bacteria in food processing environment and finally to better control food contamination from the industrial surfaces.

Methods: Biofilms formation diversity at 25°C of a B.thermosphacta strains collection (i.e. 31 strains) was characterized by using the biofilm ring test (cBRT), the crystal violet staining and the laser scanning confocal microscopy (LSCM), with the images analysis by BiofilmQ (Hartmann et al., 2021).

Results: Using cBRT, the 31 strains were classified in 4 groups, according to biofilm formation. Fourteen were classified as poor biofilm producer and represent 65% of the strains. Four strains as weak (12.5%), five as medium (15.5%) and two as high biofilm producer (6%). This shown a predominance of poor and weak early biofilm stage producer strains. The ability to form a mature biofilm was analysed with crystal violet staining. A statistical analysis shows seven significant different group of mature biofilm formation ($p<0.05$). The five higher biofilm producer groups are composed by the same 6 strains already classified as high and medium biofilm early stage producer by cBRT.

The analysis of LSCM images by BiofilmQ identified 10 parameters that influence the diversity. Biofilm are flat with high cell density, biofilm volume, biovolume, number of cells and substrate coverage.

Significance: This study is to our knowledge the first describing biofilm formation by B.thermosphacta showing 7 high biofilm producing strains.

On-site

Poster session: Biofilm ecology and ecotoxicology

Evaluation of biofilm formation by Sphingomonas and Pseudomonas strains on polyethylene and polyvinyl chloride pipe surfaces utilized in driking water distribution

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Biofilm formation in drinking water distribution systems (DWDS) leads to several operational issues. Among others, pipe material selection plays an important role in biofilm development. Recently, polymeric pipes are the most common choice to replace old pipework, as they are cheap, lightweight and easy to operate. Understanding how these materials affect microbial adhesion and subsequent biofilm formation, can be a valuable tool for biofilm control in DWDS. The objective of this study was to provide insight into biofilm formation behaviors of four strains representing microbial community in DWDS biofilms, Sphingomonas spp. and Pseudomonas spp. on surfaces of high-density polyethylene (PE100) and on unplasticized polyvinyl chloride (PVC-U). To do so, two Sphingomonas strains, Sph5 and Sph10, isolated from water treatment plant spiral wound membranes, P. extremorientalis and P. aeruginosa were cultivated in 6-well plates in the presence of coupons of the two pipe materials with R2A medium for 24 h. Developed biofilms were analyzed using fluorescence microcopy with DAPI staining, crystal violet assay and scanning electron microscopy. Liquid phase analysis allowed evaluation of bacterial fitness (OD600, Chemical Oxygen Demand degradation) and potential extracellular polymeric substances production (colloidal fractions of organics, proteins and polysaccharides). PE100 and PVC-U materials were characterized in terms of their surface topography (scanning electron microscopy), roughness (profilometry), wettability (water contact angle) and elemental composition (energy-dispersive X-ray spectroscopy, X-ray photoelectron spectroscopy). Significant differences in adhesion behavior between the tested strains were observed. While Spinhogomonas Sph5 attached as single cells, Sph10 tended to auto-aggregate and adhered in clusters creating three-dimensional layers, which accelerated biofilm formation. P. extremorientalis was the only strain producing EPS and forming multi-layer biofilm clusters after 24 h. Despite the distinct surface properties of the tested pipe materials, especially in roughness and topography, with PVC-U being much rougher than PE100, no considerable difference in adhered biomass was observed between them in the tested conditions. Showing the variety of adhesion mechanisms just for the four bacterial strains, this study illustrates the complexity of biofilm formation routes by mixed microbial communities in drinking water environments.

On-site

Poster session: Biofilm ecology and ecotoxicology

Biofilms play a role in the occurrence of unwanted microorganisms in drinking water distribution systems.

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Understanding the microbial community is important in drinking water (DW) quality management. Biofilms harbor a large fraction of these bacteria and can cause problems as they can affect taste and odor or be a potential source of bacterial contamination that can thread human health. Therefore, understanding biofilm formation and how potentially harmful bacteria grow in these biofilms and eventually go into the bulk water is essential to safeguard DW safety and quality. Despite their importance, biofilms present in distribution systems are hard to sample in practice, and thus these phenomena are poorly understood. In this work, we investigated biofilm formation in a DW network using a KIWA biofilm monitor. This continuous monitor consists of 40 glass rings that collect the biofilm. As the raw water source and the corresponding treatment contribute to the final DW quality, 2 biofilm monitors were set up for 1 year at 2 water towers that receive different types of water. First, the microbial community and physical structure of the biofilms were characterized using next generation sequencing, flow cytometry and confocal laser scanning microscopy (CLSM). This study showed that the biofilm resulting from treated groundwater has a higher viable and total cell density. Then, we determined the invasion potential of the coliform Serratia fonticola, commonly present in DW distribution systems of Flanders and beyond. After labelling this microorganism with a green fluorescent protein tag, we spiked the coliform in a vessel connected to the KIWA monitor and measured its presence in the formed DW biofilm using flow cytometry and CLSM. Our results indicate that this microorganism is able to establish itself in the biofilm. Furthermore, we identified how conditions favor the growth of Serratia fonticola in biofilms and cause the detachment of the coliform from the biofilm into the bulk water. This work showed that an increase in temperature or a change in corrosivity leads to a release of Serratia fonticola into the planktonic phase. Overall, this study will provide a novel insight into biofilm structures arisen from different types of DW. Moreover, this research will add value in understanding the relation between the planktonic community, operational conditions and the detachment of unwanted species from the biofilm, which is of utmost importance in safeguarding the DW quality.

On-site

Poster session: Biofilm ecology and ecotoxicology

The holistic approach on studying *Bacillus subtilis* pellicle development *in situ*

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Bacillus subtilis is one of the best model systems for studying biofilm formation. Although B. subtilis can form biofilms on almost any surface, most studies have focused on the biofilm formation on solid substrates. On the other hand, much less is known on *B. subtilis* biofilm forming on air-liquid interface (i.e. pellicle), typically observed in unshaken batch systems. In addition, most of the studies ignore the bacterial population in the bulk liquid phase, although it can represent a significant portion of the entire bacterial population in the batch system [1]. This prompts for a holistic approach, where pellicle formation is studied in situ, in a real-time, together with events taking place in the bulk liquid phase. We implemented this idea in our new study [2], where we were monitoring the pellicle development in *B. subtilis* PS-216 using real-time interfacial rheology and confocal laser scanning microscopy. By applying the two techniques, we elucidated the links in morphological changes and mechanical properties of the growing pellicle. The unique system view on pellicle development enabled us to correlate the events at the liquid-air interface with the vertical distribution and viability of the individual bacterial cells in the water column. We extended our approach to the mutant strains defective in exopolymeric substances production. Six key events identified in pellicle formation were marked by a major change in viscoelastic and morphology behaviour of the pellicle. The results imply that pellicle formation is a multifaceted response to a changing environment induced by bacterial growth that caused population redistribution within the system, reduction of the viable habitat to the liquid-air interface, cell developmental and morphogenesis changes, and build-up of mechanical stress supporting structure. The end of the pellicle life cycle is marked by the reduced pellicle resilience to the mechanical stress, manifesting as the pellicle collapse and the spore releasing process, indicating the system went into the dormant state.

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2.Krajnc, M., et al., Systems view of Bacillus subtilis pellicle development. npj Biofilms and microbiomes, Accepted for publication (2022).

On-site

Poster session: Biofilm applications

Counteracting Bacterial Motility: A Promising Strategy to Narrow Listeria monocytogenes Biofilm

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Listeria monocytogenes (L. monocytogenes) is an ubiquitous bacterium mainly found in water, soil, and forage. For humans, the consumption of Ready To Eat (RTE) contaminated food products represents the main transmission mode. L. monocytogenes has four to six peritrichous flagella per cell. It is able to adapt and survive to the most constraining conditions such as low temperatures, UV radiation, low pH, and high osmolarity. Flagellum-mediated motility is important for biofilm formation and in the case of L. monocytogenes, flagella are implicated as surface adhesins in early surface attachment.

There is a clear need to develop new strategies to control L. monocytogenes biofilm formation. One of the promising strategies is the discovery of new active molecules that target bacterial adhesive properties and biofilm formation without affecting bacterial viability in order to avoid the development of resistance following a life-threatening selective pressure.

This study explores the efficacy of prospect molecules for counteracting bacterial mechanisms leading to biofilm formation. The compounds included the phytomolecule tomatidine, zinc chloride (ZnCl2), ethylenediaminetetraacetic acid (EDTA), and a more complexed mixture of bacterial compounds from coagulase-negative staphylococci (CNS exoproducts). Significant inhibition of L. monocytogenes biofilm formation was evidenced using a microfluidic system and confocal microscopic analyses ($p < 0.001$). Active molecules were effective at an early stage of biofilm development (≥50% of inhibition) but failed to disperse mature biofilms of L. monocytogenes. According to our findings, prevention of surface attachment was associated with a disruption of bacterial motility. Indeed, agar cell motility assays demonstrated the effectiveness of these molecules. Overall, results highlighted the critical role of motility in biofilm formation and allow to consider flagellum-mediated motility as a promising molecular target in control strategies against L. monocytogenes.

On-site

Poster session: Biofilm applications

Bacterial biofilms as a sustainable and stable fouling control tool for submerged surfaces

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The accumulation of unwanted biological matter on surfaces, or biofouling, poses a challenge for a variety of industries. On vessels, biofouling increases drag, corrosion, fuel consumption and greenhouse emissions. Current solutions use synthetic coatings to prevent biofouling but these are constrained by their limited lifetime, efficacy and toxicity to wildlife.

Such challenge inspired our novel, biological approach using complex bacterial films, natural or engineered, as biofouling control tools of underwater surfaces. Our focus was assembling bacterial biofilm communities expressing essential traits for biofilm stability, resilience and antifouling activity in marine-relevant conditions, key for success for future technology implementation.

First, we targeted isolation of native vessel-adhering bacteria by sampling the submerged hulls of two vessels in Helsingør (Denmark). Moreover, we included 15 alga isolates (Australia) and two Roseobacter bacteria, R. denitrificans (RD) and D. shibae (DS), ubiquitous in the marine milieu. The presence of RD or DS in all tested, engineered communities is key for inserting tailored functional modules to modulate performance of the protective film. Second, we selected isolates with outperforming adhesive properties, which served as input for community assembly. Our bottom-up assembly approach involved dual-, triple- and multispecies combinations. Finally, productivity of selected multispecies communities was measured at varying temperatures (4, 10 and 24 °C) and species abundance assessed by qPCR.

We found 8 multispecies communities with highly robust and reproducible adhesion, all containing either RD or DS. Biofilm productivity and resilience was generally greater for the Helsingør communities, yielding 3-fold more biofilm at 4°C than 10 or 24°C with prolonged incubation. These constitute promising candidates for a stable protective film in such changing environment as the sea, given their adaptability to a wide temperature range (20°C). In contrast, the Australian communities showed greater cold sensitivity, with a biomass drop up to 10-fold despite the incubation time.

In summary, we assembled synthetic, stable communities that allow for further insertion of functional modules and possible application as protective films on submerged marine surfaces.

On-site

Poster session: Biofilm applications

Characterisation of anodic biofilms from microbial fuel cells based on imaging techniques

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Due to the climate change and the worsening climate crisis of the 21st century, the conservation of resources and the associated increasing demands on water quality, water treatment and water recycling have taken the centre stage. In this context, microbial fuel cells (MFC) offer an alternative and environmentally friendly development for the water treatment of heavily polluted wastewater and, at the same time, can contribute to the energy self-sufficiency through energy recovery [1].

For this purpose, exoelectrogenic bacteria are brought close to a conductive surface (anode). These microorganisms can oxidise organic substrates in the wastewater and release the electrons thus gained to the electrode. To do so, they can express their own shuttle substances such as cytochromes of type c, carotenoids or flavin molecules, which are membrane-permeable and can accept electrons. These substances migrate to the electrode via diffusion processes and are oxidised there by donating electrons. In addition, electron transfer can also be achieved through cell-cell contact or the direct attachment of the microorganisms on the anode to avoid diffusion processes [2]. In addition to electrons, protons are also released in this system, which diffuse to the cathode and are reduced to water there with the dissolved oxygen and the electrons that were previously released [1].

This work focuses on the characterisation of microbial biofilms with visualisation-based methods such as Raman microspectroscopy (RM) and scanning electron microscopy (SEM) to get a better insight into the complex composition and structure of a biofilm that is used in MFCs for the anaerobic treatment of brewery waste. This includes the 2D & 3D visualisation and in situ chemical analysis of shuttle substances, cellular components (such as proteins, lipids and polysaccharides) as well as inorganic compounds via RM. SEM provides information on the 3D structure of biofilms and their heterogeneity to complement the findings obtained by RM. Both techniques help to shed a light on the underlying mechanisms of electricity production.

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On-site

Poster session: Biofilm applications

Heavy metal bio-recovery from industrial waters: are biofilm useful?

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Electroplating industry and other anthropogenic activities represent a huge source of heavy metal wastes. Full-scale treatment of water sludges is needed not only to protect the environment, owing to heavy metal indefinite persistence and recalcitrance, but also for the economic value of their recovery. Since conventional chemical-physic treatments of metal sludges pose economic and efficiency issues, the employment of bacterial bio-sorbents as a valid alternative were studied. Specific copper, nickel and chromium removal was assessed in filter immobilized biofilm and in free planktonic biomass systems.

Environmental bacterial strains able to produce exopolymeric substances (EPS) were tested for their ability to remove copper [Cu(II)] and nickel [Ni(II)] when present in aqueous solutions. After 72 h incubation, cell biomass was either used as deposited biofilm onto a 0.2 micrometers cellulose acetate filter or directly resuspended in metal MilliQ water solution. The ability to remove different heavy metals (as analyzed by ICP-MS) was present at different extents in the analyzed strains. When used in biofilm system, Serratia plymuthica strain As3-5a removed 48% of metal from a 200 mg/L copper solution; interestingly, the removal decreased to 34% when doubling the biomass. When used in planktonic cell system, As3-5a strain removed up to 91% of total copper. The same behavior was observed for different strains. Serratia mygulae strain SC3I(2) removed 52% of metal from a 50 mg/L nickel solution when in biofilm, and up to 89% when in planktonic cell system; for this strain the use of higher biomass onto the filter did not altered the sorption capacity. Further analyses are running in order to fit the data in adsorption kinetics models.

In previous works, Serratia marcescens strains have been considered for their ability to remove toxic metals. Considering the high bio-sorption ability of the Serratia strains tested in the present study, it possible to envisage their use as bio-sorbing platforms to segregate heavy metals out of industrial wastewaters. The data indicated also that cell EPS metal binding sites were probably masked due to superposition of cells in the biofilm and their use in packed bed biofilters needs deeper comprehension of the system.

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On-site

Poster session: Biofilm applications

Polyanionic property of extracellular polymers from seawater adapted aerobic granular sludge as a potential application for sepsis treatment

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Sepsis is an infectious disease causing a hyperinflammatory response to the body's organs and tissue. Currently, no specific treatments are available for sepsis. Extracellular histones play a major role in the death of sepsis patients. Negatively charged polymers, like heparins and polysialic acids, have shown potential to treat sepsis by neutralizing the cationic histones and thus blocking histone cytotoxicity. Polymers containing sialic acids have been detected in the extracellular polymeric substances (EPS) of seawater-adapted aerobic granular sludge (AGS). However, the anionic fraction has not yet been enriched and further identified. This study aims to enrich the anionic fraction of the EPS and assess its potential application in binding cationic histones. Seawater-adapted AGS was cultivated in a lab-scale reactor on synthetic wastewater and the EPS was extracted under alkaline condition. The anionic fraction of the EPS was first enriched by a protease treatment followed by anion exchange chromatography separation, purified by DNase digestion, and analyzed by mass spectrometry. The interaction between the enriched EPS with the histones was visualized by native agarose gel electrophoresis with Coomassie blue staining. It was found that high levels of sialic acids and –OSO3- were present in the EPS, which contributed to its polyanionic property. The enriched polyanionic EPS fractions successfully neutralized the positive charge of histones and inhibited their migration in the agarose gel. This indicated that the enriched polyanionic EPS extracted from seawater-adapted AGS has the potential to be developed as sepsis treatment by binding the extracellular histones.

On-site

Poster session: Biofilm applications

Microalgae against climate change – a science outreach project

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Modern research has so been given little public attention so far. This is mainly due to the fact that the scientific issues are often presented in an incomprehensible way to those outside the scientific community. At the same time, there is an increasing desire on the part of civil society for information and participation in subject-related research. This is particularly true regarding sustainability issues such as the implementation of the sustainable development goals (SDGs). To date, however, there are few research-based statements on how to best involve the non-scientific public. However, one important requirement for science outreach is well known: Only the intensive cooperation between scientists from the subject-related disciplines and the corresponding subject didactics can ensure that the subject-related research topics are available to the non-specialist public in an optimal form. In the context of Science Outreach offers (SDG 4 "high quality education"), the potential of the use of microalgae in relation to climate protection (SDG 13 "Climate protection") will be demonstrated and, at the same time, the conditions for the success of Science Outreach will be determined.

Initially, a plug-in bioreactor system for the cultivation of microalgae will be developed in the subject-related part of the project and methods of cultivation will be further optimized. In addition, an environmentally friendly method of extracting pigments from microalgae will be developed. This should allow scientific knowledge on the production of dyes such as phycocyanin and chlorophyll-a to be presented in educational projects. The system should make it possible to compare process parameters such as different reactor geometries or cultivation in the submerged and surface associated systems in a do-it-yourself experiment. In the field of didactics, didactic-methodical materials and concepts such as (digital) experiments and educational videos to the topic of microalgae and their use will be developed. The focus will always be on the aspect of sustainability and the link to the SDGs. The materials created will be used in various science outreach activities on the topic of "Microalgae against climate change". In this study, the latest results of the development of the plug-in photobioreactor system and the environmentally friendly extraction and separation of photopigments of various microalgae are presented.

On-site

Poster session: Biofilm applications

Scale-up of capillary photo-biofilm-reactors

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The use of phototrophic cyanobacteria in biotechnology is highly interesting as they represent a carbon neutral production platform, relying mainly on carbon dioxide, light and water for growth. However, one key bottleneck for utilizing cyanobacteria as production hosts is the low cell density of only 2 to 4 gCDW/L in established cultivation systems. One promising concept to solve this shortcoming is the cultivation as dual trophies biofilms in microtubular systems in a segmented flow fashion [1]. Scaling is possible via increase of capillary length and parallelization of the single reactor units [2, 3]. Increasing capillary length challenges resource management, especially when working with single point feeding strategies, whereas parallelization demands equal and stable hydrodynamic conditions throughout the system [3].

The efficient removal of oxygen from the reactor system is essential, as high oxygen concentrations inhibit the photosystems of Synechocystis sp. [4]. Thus, silicone as reactor material improved oxygen removal from the system. In order to evaluate scaling options for this system, the length of the tubes was increased 25-fold from 0.2 m to a final length of 5 m. With an increase of the flow rate from 52 µL/min to 260 µL/min and an increase in the concentration of trace elements, a homogeneous surface coverage was observed without any biofilm detachment events (sloughing) occurring. The final measured biomass dry weight was 87.4 g/ m2.

In a subsequent step, we operated 6x5 m-biofilm reactors in parallel in a field experiment. For a more efficient use of resources, the medium was filtered, recycled and mixed with fresh medium in a ratio of 1:1. However, the biofilm growth was hampered by a precipitation of trace elements in the medium with 5-times increased trace element concentrations.

In conclusion, we could show that a scale-up of the capillary reactor system from 0.2 m to 5 m is possible. To manage the resources added to the reactor system, the optimal medium composition in terms of concentrations seems to be critical. In single use, too much nutrients go to waste; therefore, a recycling strategy has been tested, but careful tuning of the growth medium is still necessary to lead to a more economic use of resources.

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On-site

Poster session: Biofilm applications

Physiological transition of Chlorella vulgaris from planktonic to immobilized conditions

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The interest by the biofilm-based technologies is remarkably increasing due to their advantages compared to the conventional planktonic systems (e.g., higher productivity, lower water demand, low harvesting costs, …) for microalgae cultivation. Though promising, a better understanding of biofilm formation mechanisms is still required to develop and run these systems at large-scale. Among them, the physiological transition from planktonic to immobilized state has never been studied for microalgae before. Here we tracked the changes in growth and physiology (i.e. cell size, photosynthetic performance, carbon, nitrogen and Chl a quotas and macromolecular composition) of Chlorella vulgaris during the transition (over 24 hours) from planktonic to immobilized conditions. Results clearly confirmed that microalgae rapidly respond to the new conditions via physiological adjustment. Moreover, this behavior is specific to cells in the immobilized state. Very rapidly, cells use photosynthesis to grow (increase in size) and adjust the carbon allocation (increase in the relative carbohydrates pool). Triggering factors such as light, water availability, quorum sensing may induce this fast acclimation process.

Poster Abstracts

Online poster session 1

9th May 3 - 4 pm

Biofilm ecology and ecotoxicology

Biofilm application

Online

Poster session: Biofilm ecology and ecotoxicology

Toward understanding of the role of iron in Legionella pneumophila behavior in the specific context of cooling towers

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Legionnaires' disease is a human respiratory infection caused by *Legionella spp*. bacteria. These bacteria are basically found in freshwater environments including lakes and streams and they find a favourable growing environment in all human-made building water systems. The disease does affect more than 10 000 persons per year in Europe and the reported cases increased in recent years from 1,4 in 2015 to 2,2 cases per 100 000 population in 2019. In addition to the direct impact on infected people, Legionnaire's disease impacts on health systems and reduces global productivity with a huge estimated economic burden at 1 billion per year in Europe. As with other bacterial pathogens, future control will be also compounded by the continued emergence of antibiotic resistance, so it is imperative to develop new ways to understand and control *Legionella spp*. growth and spread.

Legionella is a chameleon able to persist in the environment in a free-living planktonic form but can also make biofilm to adhere and colonise surfaces. Bacterial pathogenesis is directly related to its facultative intracellular lifestyle and its ability to infect human cells.

Iron has been described as an essential nutrient for *Legionella spp*. growth and replication. However, the role of iron in *Legionella* biofilm development is controversial, as well as the role of iron in bacterial persistence and growth within its host cells.

In our study, we have developed an iron electrochemical sensor we can use to monitor the presence and quantity of iron in both ferrous Fe(II) and ferric Fe(III) states. Using this newly developed tool, we have unravelled a difference in the role played by the two iron forms on *Legionella pneumophila* behaviour. In environmental conditions, the ferrous Fe(II) iron is oxidized in ferric Fe(III) iron, being then the state impacting the most, if not the only one, on bacteria. Ferric Fe(III) iron is responsible of a decrease in *Legionella pneumophila* population (viability and growability of bacteria) within the biofilm but it does not inhibit biofilm formation. Also, the difference observed in between the decrease of cultivable bacteria (CFU) and the lower decrease of the total number of bacteria (genome copy number) suggests the presence of viable but non-culturable (VBNC) bacterial form. These data shed light on the potential key role of iron in *Legionella pneumophila* behaviour associated to pathogenesis.

Online

Poster session: Biofilm ecology and ecotoxicology

Mechanistic action of weak acid drugs on biofilms

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Biofilm matrix are highly viscoelastic in nature and this property is thought to play a key role in protection of biofilms from toxin and other invaders. Selective permeability of a biofilm matrix to some drugs has resulted in the development of drug tolerant bacteria. The efficacy of a weak organic acid drug, N-acetyl-L-cysteine (NAC), on the eradication of biofilms and the commonality of NAC with that of acetic acid have been investigated. NAC and acetic acid at pH < pKa penetrate biofilm matrix and eventually kill 100% of the bacteria embedded in a variety of biofilms with different matrix composition, including antibiotic resistant strains. Once the bacteria are killed, the remnant matrix swells in size and passively shed bacteria, suggesting that the bacteria act as crosslinkers within the extracellular matrix Despite shedding of the bacteria, the remnant matrix of mucoid P. aeruginosa biofilms remains intact and behaves as a pH-responsive hydrogel that swells and shrinks with changes in pH. Correspondingly, the matrix rheological properties showed that the remnant matrix maintained its elastic properties and had the response of an ultra-soft polyelectrolyte hydrogel.

Online

Poster session: Biofilm ecology and ecotoxicology

Regulation of Pseudomonas putida biofilm by extracellular peptides and LapA domain vWFa

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The biofilm of Pseudomonas putida is complexly regulated by several factors. The focus has been on the bacterial self-encoded factors such as the adhesins LapA and LapF, the regulators GacS/A, FleQ, Fis, and the signalling molecules c-di-GMP, pp(p)Gpp, and so on. Less attention has been paid to environmental factors that may influence biofilm formation and development. We noticed that in the presence of peptides in the medium, the biofilm of P. putida is enhanced. Consequently, we asked whether peptides promote biofilm as a source of C and N or as an architectural component. We ascertained that peptides in the medium could promote P. putida biofilm as an architectural component. Although P. putida wild type strain PSm has peptide-dependent biofilm enhancement, it was more pronounced in P. putida strain F15, where global regulatory fis expression was elevated by IPTG. Since Fis activates lapA transcription, we hypothesized if LapA could mediate the enhancing effect of Fis and peptides. Moreover, LapA of Pseudomonas fluorescens has several domains that could be involved in the surface attachment; we focused on the LapA vWFa domain. This domain is similar to von Willebrand Factor domain A, which is described as a domain similar to von Willebrand factor domain A, which is involved in the formation of blood clots by binding peptides. We showed that LapA must have a functional vWFa domain to reveal the enhancing effect of extracellular peptides. Moreover, the Fis biofilm enhancing effect is related to the LapA vWFα domain.

Online

Poster session: Biofilm ecology and ecotoxicology

Electrotrophic organication of carbon and nitrogen in cathodic biofilms

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The electrotrophic production of bacterial biomass represents a challenging opportunity with several industrial applications in terms of organic carbon storage and soil fertility improvement. To evaluate the electrotrophycally driven biomass development, an environmental microbial consortium was inoculated in a bio electrochemical system (BES) where only N2 and inorganic carbon were provided. The experimental set-up included three BESs inoculated with microbial consortium and operated at a constant cathodic potential of −0.7V vs SHE. Those were compared with four control systems: one BES inoculated with microbial consortium and operated at a constant cathodic potential of −0.2V vs SHE; one BES not inoculated and operated at a constant cathodic potential of −0.7V vs SHE; two BESs inoculated with microbial consortium and operated at open circuit (OC). After an initial period of stabilization, all seven BESs were run for 165 days.

Preliminary results showed a higher charge consumption in the inoculated systems at -0.7V polarization compared with the controls. At the end of incubation, polarized inoculated systems produced a higher biomass in the cathodic chamber compared with OC. DNA and RNA were isolated from the cathodic biofilm, the microbial suspension and the bottle wall biofilm and microbial consortia are under characterization by Illumina 16S rRNA gene sequencing and by shotgun sequencing.

These outcomes offer new insights on the ecological and physiological mechanisms involved in the establishment of microbial consortia in oligotrophic environments, and pave the way for novel biotechnological applications.

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Online

Poster session: Biofilm ecology and ecotoxicology

Anti-bacterial Susceptibility and Biofilm Forming Ability of Foodborne Pathogens Isolated from Minimally Processed Fruits and Vegetables Obtained from Markets in Southeastern Nigeria.

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The resistance of foodborne pathogens to antibiotics and their ability to form biofilms poses a serious public health burden globally. This study investigated the antibacterial susceptibility pattern and biofilm-forming ability of bacterial isolates from surfaces of minimally processed fruits and vegetables from markets in Southeastern Nigeria.

Randomly selected samples of fresh minimally processed watermelon, cucumber, and garden egg were collected in July, 2019 and evaluated by standard plate techniques. Pure cultures were identified macroscopically, microscopically, and using biochemical analysis. The antibiotic susceptibility studies were conducted using the Kirby-Bauer disk diffusion method on eleven antibiotic discs. The biofilm screening was conducted using Congo Red Agar medium and scanning electron microscopy (SEM) was to determine morphological features of biofilm formers.

Characterization revealed seventeen probable species of pathogenic bacteria. Antibiotic susceptibility results revealed MTX50 having the least antibacterial activity with percentage susceptibility of 0 and AMP10, the most effective antibiotic on all isolates with percentage susceptibility of 70.41. Salmonella sp. had a mean IZD of 18.67 mm to PEF5 and according to CLSI guidelines, it is said to be resistant to PEF5. All isolates were strong biofilm formers except four which were non- and moderate biofilm formers. SEM micrograph revealed organisms enclosed in an extracellular matrix. According to WHO and Food and Agricultural Organization, standard values for microbial colonization should not exceed 10^5cells/ml for total aerobic bacteria, 10^3cells/ml for enteric bacteria, and salmonella and E.coli should totally be absent. The total viable count of probable bacteria (8.0×10^6 to 1.0×10^6) from all samples exceeded the standard limit and samples also harboured salmonella and *E.coli*.

The detection of these opportunistic pathogens capable of forming biofilms in freshly minimally processed fruits and vegetable poses a serious risk for consumers because of their bacteria colonization indication ratio and resistance patterns which varied in response to the various antibiotics used. Biofilm formers indicate resistant pathogens as they formed extracellular polymeric matrices.

Keywords: Antibacterial susceptibility, microbial contamination, biofilm formation, food-borne pathogens, vegetables, fruits.

Online

Poster session: Biofilm applications

Anti Quorum Sensing, Anti Virulence, Anti Biofilm and NF-κB Inhibitory Activities of Dihydroxy Piperlongumine (PL-18), a Derivative of Piperlongumine from Piper longum

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Bacterial communication, termed Quorum Sensing (QS), is a promising target for virulence attenuation, thus providing an alternative treatment to decrease antibiotic-resistant infections. In this work, six amide alkaloid derivatives of piperlongumine (PL) from Piper nigrum were screened for both, QS and Nuclear Factor kappa B (NF-κB) inhibition. As a result of the screening, PL-18 was identified and further investigated. PL-18 has two hydroxy groups. It is obtained from PL by demethylation. Two of the three methoxy groups of PL were demethylated to get PL-18. The presence of hydroxy groups is significant in regard to QS inhibitory (QSI) activity. QSI activity was determined on Agrobacterium tumefaciens (KYC55) and Chromobacterium violaceum (CV026) reporter bacteria and against the clinically relevant Pseudomonas aeruginosa; both against lasR-lasI and rhlR-rhlI P. aeruginosa QS systems by the reporter PAO-J2 strains and by RT-qPCR of the PA01 strain.

Pyocyanin, rhamnolipids and the buildup of biofilm are virulence important elements produced by P. aeruginosa. Their expression is controlled by QS. These three elements were significantly inhibited by PL-18. Interestingly, PL-18 is not antibiotic against P. aeruginosa.

NF-κB is a pro-inflammatory transcription factor that is upregulated in many inflammatory diseases, including those induced by bacterial infections. NF-κB inhibition experiments were performed on L428 cells transfected with the luciferase NF-κB reporter gene and on A549 cells, by performing immunofluorescence stains for in-situ localization of p65-NF-κB. Similarly to PL, PL-18 also efficiently inhibited NF-κB activation. The substitution of the methoxy groups by hydroxy groups did not abolish the NF-κB inhibitory activity. Nevertheless, in agreement with previous work, different PL derivatives where the alkaloid ring was modified, resulted in the abolishment of their anti inflammatory activity. Furthermore, PL-18 was found to be cytotoxic to transformed cell lines. QS inhibition is a promising approach to prevent bacterial virulence by disturbing bacterial communication. Coupled with the ability to inhibit NF-κB induced inflammation, PL-18 is a suitable candidate as a potentially effective compound against bacterial infection, antibiotic resistance and biofilm formation.

Online

Poster session: Biofilm applications

Biofilms components plays important role in interspecies interactions between *Bacillus subtilis* and *Salmonella enterica*

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The influence of social interactions on the spatial distribution of different species in biofilms can affect the fitness of interactants, which is particularly relevant for mixed biofilms of a potential probiotic and a pathogen. Here, we focus on the sociality between *Bacillus subtilis* and *Salmonella enterica* and how the extracellular matrix components of *S. enterica* affect the competitiveness, adhesion, and spatial separation of both species.

Biofilms of *S. enterica* pose a major challenge to food safety in the food industry. Its extracellular matrix is mainly composed of curli fimbriae (amyloid fimbriae), cellulose, biofilm-associated protein (BapA), O-antigenic capsule, and extracellular DNA. To address the role of matrix components in interspecies interactions, we focused on the role of cellulose and curli and how their presence/absence affected the adhesiveness and biofilm thickness of *S.* enterica in the presence and absence of the potential probiotic strain *B. subtilis* PS -216. All strains produced constitutively fluorescent proteins and carried antibiotic markers, allowing us to monitor their abundance and spatial distribution in co-cultures by confocal microscopy. The results show that *B. subtilis* PS -216 decrease the fitness of *S. enterica* and that both indicated matrix components affect the competitiveness but not the distribution of Salmonella in coculture with *B. subtilis*. Furthermore, csgB deletion negatively affected the fitness/biofilm thickness of *S. enterica* in coculture, whereas the mutant lacking cellulose showed improved fitness/biofilm thickness in the presence of *B. subtilis*. This could be an important clue for the development of efficient removal procedures